

# Effects of elicitors on the growth and active compounds of *Panax vietnamensis* cells in the bioreactor

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## **Abstract:**

In this study, the effects of elicitors on the growth and bioactive ingredients of *Panax vietnamensis* cell mass are evaluated. The medium composition was fixed with a MS liquid medium supplemented with 6.42 mg/ml NAA, kinetin of 0.11 mg/l, saccharose of 34.3 g/l, a pH of 5.6-5.8, temperature of 24-26°C, conducted at 50 rpm, and culture time of 14 days. The results showed that the best elicitor is jasmonic acid, at a concentration of 100  $\mu$ mol/l, and for an optimal exposure of the bioactive ingredients to jasmonic acid for 12 days and 14 days after starting the culture. Under select conditions, the cell mass yield was 20.01 g/l and contents of ginsenoside Rg1, Rb1, and Rd were 0.288, 0.302, and 0.146%, respectively.

**Keywords:** biomass, MS, *Panax vietnamensis*, saponin.

**Classification number:** 3.3, 3.5



150-year-old roots of *Panax vietnamensis*.

## **Introduction**

Ngoc Linh Ginseng (*Panax vietnamensis* Ha et Grushv.) belongs to the Araliaceae family, and is a unique and valuable herb found on Ngoc Linh Mountain. Currently, this species has been included in the Red Book of Vietnam. Natural Ngoc Linh Ginseng only grows at altitudes above 1,500 meters, mainly concentrating in the Ngoc Linh Mountain area of the Dak To (Kon Tum) and Tra My (Quang Nam) Districts. Ngoc Linh Ginseng has many biological uses including nutritious, tonic, antioxidant, anti-aging, and hepatoprotective effects. Ngoc Linh Ginseng is a rare and valuable medicinal plant, which has been exhaustively exploited [1, 2].

Today, plant cell biomass technology has been widely applied in many fields of pharmaceuticals, cosmetics and functional foods. Vietnam Military Medical University (VMMU), together with Ajou University in South Korea, has been successfully carrying out collaborative projects, namely: *Research collaboration on the development of the production procedure for Vietnamese ginseng (Panax vietnamensis) biomass as a raw material for health products to serve publics* (2007) [3]. This collaborative project has been highly appreciated by the scientific committee and has been recommended for further progress to develop a complete biomass production procedure of Ngoc Linh Ginseng cells. Based on the results of the research project, VMMU have been granted a patent for the methodology for a Ngoc Linh Ginseng biomass production procedure by the Intellectual Property Department of the Vietnamese Ministry of Science and Technology (2009) [4]. AS a result of the above mentioned success, scientists from VMMU continue to carry out this ministry-level project, now titled the *Study on the Vinantonic preparations from Vietnamese ginseng cellmass*, this product has been

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approved by the Ministry of Health and is now circulated nationwide [5].

Based on the results of these successful projects, the Ministry of Science and Technology has agreed to allow VMMU to carry out a national-level project: “*Ngoc Linh Ginseng cellmass production process optimization and Vinatonic preparation*” [6]. To implement this project, a study of conditions, especially oxygen concentration for optimized cell biomass grown in 100 liter bioreactors, is important. In this article, we evaluate the effects of oxygen concentration on the development and content of active ingredients in Vietnamese ginseng cell biomass in 100 liter bioreactors.

### Experimentals

#### Instrument, reagents

- Instrument: Incubator 2 NU-440-400E, Orbital shaker, Incubator ISF4-W, 100 liter Bioreactor Biostat C200 (Germany).

- Reagents: Myo-inositol, Nicotinic acid, Glycine, Pyridoxine hydrochloride, Thiamine hydrochloride, Kinetin, Glucose, Sucrose, Acid 2.4 - dichloro phenoxy acetic, Kali nitrate, Amoni nitrate, Magnesium sulfate, Kali dihydro phosphate, and other reagents that meet culture standards.

#### Material and methods

**Material:** *Panax vietnamensis* cellmass (provided by the Biomedical and Pharmaceutical Applied Research Center at VMMU).

**Methods:** Cultured *Panax vietnamensis* cellmass was studied in an optimal medium, including: (MS: Medium supplemented with 6.42 mg/l of NAA; 0.11 mg/l of kinetin; 34.3 gram of saccharose in 1 liter of culture medium). Different elicitors were used to increase the content of bioactive compounds in the ginseng cellmass. After each culture batch, the ginseng cell was filtered. Then the dry cellmass and quantify ginsenoside in the cellmass were weighted. After analyzing the results, the best elicitor for cellmass and bioactive compounds was selected.

- Select suitable elicitors: Different elicitors were used including jasmonic acid, ferulic acid, caffeic acid, methyl jasmonate, and yeast extract. These elicitors were added on the 12th day of the culture cycle. After finishing the culture cycle, the cellmass was filtered, collected, and quantified as the bioactive compounds in dry cellmass. From

**Table1. Results of elicitor selection.**

Elicitor	Ginsenoside (%)			Dried cells weight (g/l)
	Rg1	Rb1	Rd	
Without elicitor	0.2521±0.010	0.2722±0.012	0.1430±0.005	20.26±2.05
Jasmonic acid	0.3432±0.013	0.3847±0.019	0.1711±0.007	18.58±1.88
Methyl jasmonate	0.3164±0.013	0.3441±0.014	0.1597±0.005	18.40±1.88
Ferulic acid	0.2583±0.009	0.2529±0.010	0.1259±0.004	19.19±2.14
Caffeic acid	0.2469±0.010	0.2878±0.014	0.1315±0.005	19.60±2.26
Yeast extract	0.3096±0.012	0.3536±0.014	0.1838±0.007	18.60±2.28

the results, the best elicitor was selected.

- Select optimal elicitor concentration: After 12 days of the culture cycle, ginseng cells were exposed to jasmonic acid at different concentrations: 50, 100, 150, 200, 250 and 300 µmol/l; and allowed to continue to culture for two more days. The cellmass and quantitated active compounds in cellmass were then collected. From the results, the best concentration of jasmonic acid was selected.

- Select the suitable time of adding the elicitor: Jasmonic acid was added at a concentration of 100 µmol/l to the culture medium on the 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup>, 9<sup>th</sup>, 11<sup>th</sup>, 12<sup>th</sup>, 13<sup>th</sup> and 14<sup>th</sup> day of the culture cycle. Two days after adding jasmonic acid, the ginseng cells were wilted quantitated ginsenoside content in the dry cellmass. From the results, the best time to add an elicitor was identified.

- Select the optimal exposure time for the elicitor: On the 12<sup>th</sup> day of culturing the ginseng cells, the cells were exposed to jasmonic acid at a concentration of 100 µmol/l, over 12, 24, 36, 48, 60 and 72 hours after exposing the cells to the elicitor. The cells were then filtered, dried and quantified to measure the content of ginsenoside. From the results, the optimal exposed time to the elicitor was selected.

### Results and discussions

#### Selecting elicitor types

In order to culture the ginseng cellmass in optimal MS medium, on the 12th day of culturing, the cells were exposed to jasmonic acid at a concentration of 100 µmol/l; methyl jasmonate (100 µmol/l), ferulic acid (100 µmol/l), caffeic acid (100 µmol/l), and yeast extract (100 µg/g fresh weight). 48 hours after exposing the cells to the elicitor, the cells were filtered, dried to constant weight, and

quantified as to the content of ginsenoside. Results are presented in Table 1.

When elicitors were used, the content of ginsenoside Rb1, Rg1, Rd was enhanced compared to those of the control. Among elicitors used, jasmonic acid, methyl jasmonate, and yeast extract showed the best results.

Experimental results showed that, jasmonic acid, methyl jasmonate, and yeast extract can be used for the cultivation of Vietnamese ginseng roots cellmass as elicitors in order to increase the synthesis of bioactive compounds to enhance the saponin content of the final products. For further studies, jasmonic acid was used.

Table 1 shows that when an elicitor was used, the dried cellmass was lower, compared to that of the control group; thus elicitors stimulated the synthesis of active compounds, at the same time reducing the ginseng cellmass growth. Our results were similar to other published results of plant cell biomass, and this was explained by the elicitors as well as strange agents, and pathogens, when exposed to cells, inhibited the growth of ginseng cells. In response to that observation, the cells have a defense mechanism to stimulate the synthesis of phytoalexin (almost all active compounds are phytoalexin).

The effects of elicitor concentrations on the content of bioactive compounds in Vietnamese ginseng cellmass in 100 bioreactor liter showed the culture of the ginseng cellmass in optimal MS medium on the 12th day of culturing, the cells were exposed to jasmonic acid at different concentrations at 50 µmol/l, 100 µmol/l, 150 µmol/l, 200 µmol/l, 250 µmol/l and 300 µmol/l. The contents of ginsenosides in dry cellmass are shown in Table 2.

**Table 2. Results for selection of elicitor concentrations.**

Concentration ( $\mu\text{mol/l}$ )	Ginsenoside (%)			Dry cell weight (g/l)
	<i>Rg1</i>	<i>Rb1</i>	<i>Rd</i>	
0	0.2512 $\pm$ 0.012	0.2709 $\pm$ 0.011	0.1421 $\pm$ 0.005	20.22 $\pm$ 2.33
50	0.3032 $\pm$ 0.010	0.3308 $\pm$ 0.013	0.1544 $\pm$ 0.007	19.26 $\pm$ 2.36
100	0.3494 $\pm$ 0.013	0.3852 $\pm$ 0.016	0.1705 $\pm$ 0.006	18.63 $\pm$ 2.25
150	0.3470 $\pm$ 0.015	0.3890 $\pm$ 0.014	0.1728 $\pm$ 0.006	18.15 $\pm$ 2.26
200	0.3311 $\pm$ 0.012	0.3283 $\pm$ 0.014	0.1614 $\pm$ 0.007	17.87 $\pm$ 1.89
250	0.3290 $\pm$ 0.011	0.2761 $\pm$ 0.009	0.1559 $\pm$ 0.008	17.06 $\pm$ 1.73

**Table 3. Influences of adding-time of an elicitor on cellmass and ginsenoside.**

Days	Ginsenoside (%)			Dry cell weight (g/l)
	<i>Rg1</i>	<i>Rb1</i>	<i>Rd</i>	
1	0.0892 $\pm$ 0.004	0.1032 $\pm$ 0.004	*	12.74 $\pm$ 1.42
4	0.1201 $\pm$ 0.004	0.1181 $\pm$ 0.005	*	12.64 $\pm$ 1.45
6	0.1702 $\pm$ 0.006	0.1296 $\pm$ 0.006	0.0518 $\pm$ 0.002	13.02 $\pm$ 1.59
8	0.2015 $\pm$ 0.008	0.1795 $\pm$ 0.006	0.0540 $\pm$ 0.002	15.39 $\pm$ 1.86
10	0.2864 $\pm$ 0.010	0.3086 $\pm$ 0.013	0.1281 $\pm$ 0.006	17.94 $\pm$ 2.23
12	0.3408 $\pm$ 0.014	0.3624 $\pm$ 0.013	0.1479 $\pm$ 0.005	18.25 $\pm$ 1.93
14	0.3495 $\pm$ 0.017	0.3838 $\pm$ 0.016	0.1714 $\pm$ 0.007	18.58 $\pm$ 1.92
16	0.3290 $\pm$ 0.012	0.3686 $\pm$ 0.012	0.1629 $\pm$ 0.005	17.66 $\pm$ 1.79
20	0.2506 $\pm$ 0.010	0.2707 $\pm$ 0.009	0.1430 $\pm$ 0.005	16.95 $\pm$ 1.74

\*Undetected.

**Table 4. Effects of exposed time to an elicitor on the content of ginsenoside and cellmass.**

Exposed time (hours)	Ginsenoside (%)			Dry cell weight (g/l)
	<i>Rg1</i>	<i>Rb1</i>	<i>Rd</i>	
12	0.288 $\pm$ 0.010	0.302 $\pm$ 0.011	0.146 $\pm$ 0.005	20.01 $\pm$ 0.66
24	0.306 $\pm$ 0.011	0.308 $\pm$ 0.013	0.148 $\pm$ 0.006	19.66 $\pm$ 0.68
36	0.325 $\pm$ 0.013	0.341 $\pm$ 0.011	0.160 $\pm$ 0.008	19.21 $\pm$ 0.76
48	0.351 $\pm$ 0.017	0.381 $\pm$ 0.013	0.174 $\pm$ 0.006	18.52 $\pm$ 0.64
60	0.316 $\pm$ 0.011	0.331 $\pm$ 0.014	0.152 $\pm$ 0.006	17.30 $\pm$ 0.69
72	0.275 $\pm$ 0.009	0.290 $\pm$ 0.010	0.136 $\pm$ 0.005	15.31 $\pm$ 0.74

Results in Table 2 show that, when jasmonic acid was used at the concentration of 100  $\mu\text{mol/l}$ , the content of ginsenoside in dry cellmass was the highest, so this concentration was selected as a suitable concentration for culturing the Vietnamese ginseng.

*Selecting of the suitable time and duration to add an elicitor*

Culturing ginseng cells in the optimal MS medium supplemented with jasmonic acid at a concentration of 100  $\mu\text{mol/l}$  on the 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup>, 9<sup>th</sup>, 11<sup>th</sup>, 12<sup>th</sup>, 13<sup>th</sup> and 14<sup>th</sup> day of the culture cycle, is optimized after two days of the addition of jasmonic acid. The cells were filtered and dried to determine the weight of the cellmass and content of ginsenosides in the cellmass. Results are shown in Table 3.

#### *Selecting the optimal exposed time to an elicitor*

On the 12<sup>th</sup> day of culturing ginseng cells, the cells were exposed to jasmonic acid at a concentration of 100  $\mu\text{mol/L}$ , from 12, 24, 36, 48, 60 and 72 hours after exposure to an elicitor, the cells were filtered, dried and quantified the content of ginsenoside. Results are shown in Table 4.

Results show that the optimal exposure time to jasmonic acid was 48 hours (two days), when the content of ginsenosides in the cellmass was the highest.

#### Conclusions

In this study, the effects of elicitors on the growth and bioactive ingredients of *Panax vietnamensis* cellmass were evaluated. The best elicitor was jasmonic acid, the best concentration of jasmonic acid was 100  $\mu\text{mol/l}$ , and the optimal time of exposure to jasmonic acid was 12 days and 14 days after starting the culture. With select conditions, the cellmass yield was 20.01 g/l and the contents of ginsenoside *Rg1*, *Rb1* and *Rd* were 0.288, 0.302 and 0.146 %, respectively.

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